SUNFLOWER RESISTANCE TO RACE G OF BROOMRAPE: THE DEVELOPMENT OF THE LINES AND THE STUDY OF INHERITANCE

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Abstract

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Introduction.

Malicious weed broomrape (*Orobanche cumana* Wallr.) is an obligate parasite from higher flowering herbaceous plants, which causes significant damage to the sunflower crop in the most countries where it is cultivated. The intensification of sunflower cultivation as a high-yielding crop has resulted in the emergence and rapid spread of new highly virulent races of the parasite that overcame the immunity of sunflower varieties and hybrids that were previously resistant to it.

The monitoring of the racial composition of broomrape, carried out by us every year, has showed that race G already prevails in most populations of broomrape in the southern regions of the Russian Federation [1, 2]. Therefore, the search for sources of immunity to this race in order to develop a resistant initial material of sunflower and to study the genetic control of this trait is of high priority.

The aim of this work was to search for possible sources of resistance, the development on their basis sunflower lines that are not affected by race G of broomrape, and the determination of genetic control of this trait.

Materials and methods. Seeds of broomrape were collected on the fields of the Bokovskiy district of the Rostov region. The identification of their racial belonging with the help of the known differentiation lines: 202A (C), LC1002 (D), LC1003 (E), LC1093 and P96 showed that the seeds belong purely to the race G. The material for research were over 1000 samples of cultivated sunflower from the collection of the Kuban Experimental Station VIR, the collection of VNIIMK of cultivated sunflower of various origin, as well as the breeding lines VK 551, VK 678 B, VK 678 A, VK 1 IMI B, VK 1 IMI A, VK 301, VK 580 and PRO2, susceptible to broomrape.

Forced self-pollination and hybridization of sunflower plants were carried out using the method, which is customary for VNIIMK [3]. A greenhouse evaluation of resistance to broomrape was carried out by the method of A.Y. Panchenko [4]. To create an infectious background in the greenhouse, the broomrape seeds were added to the boxes with soil-sand mixture at the rate of 200 mg per 1 kg of mixture, distributing them evenly. Sunflower plants were grown at a temperature of 25-27 ^{0}C and a 16-hour photoperiod. The plants were digged out in 25 days after the seedlings emergence and the broomrape specimens on their roots were counted. VNIIMK 8883 was used as a control, which is susceptible to the modern races *O. cumana*.

Analysis of variance of the received data was carried out according to the method laid down by B.A. Dospekhov [5]. Chi squared analyses were carried out to detect the deviations from the expected Mendelian ratios 1:2:1 or 1:1 [6].

Results and discussion. Over the last century of the joint evolution of sunflower and *O. cumana* plants there has been a constant rapid adaptation of the parasite to the mechanisms of host immunity. Consequently, the detection of resistance genes in cultivated sunflower has become problematic. Thus, most of the studied samples were affected to a high degree, some at the susceptible control level. However, samples were found that were divided into weakly affected and non-affected genotypes, further work with which can make it possible to develop lines immune to race G. The selection of such samples of domestic and foreign sunflower from the VIR collection are listed in Table 1. Among the presented material, samples of local breeding from the Krasnodar Region attract attention: lines VIR-665, VIR-221, VIR-222, Kabardino-Balkarian (No. 667 catalog), Armenian (No. 769 catalog), Argentine (No. 3046 catalog).

Table 1. The degree of infestation by race G of broomrape (*O. cumana*) of some samples of cultivated sunflower of VIR collection in greenhouse conditions

Catalog No.	Origin	Number of evaluated plants, pcs.	Infested plants, %	Degree* of infestation
667	Kabardino-Balkaria	30	23,3	2
769	Armenian SSR	30	23,3	1
1010	England	30	80,0	5
3300	The Krasnodar region, line VIR-221	30	33,3	4
3475	The Krasnodar region, line VIR-665	30	10,0	2
2005	The Primorsk region	26	26,9	2
3109	Bulgaria	23	17,4	2
3301	The Krasnodar region, line VIR-222	30	50,0	3
1434	Bulgaria	28	60,7	6
3046	Argentina	22	9,0	2
2954	Argentina	30	80,0	12
2925	France	30	100	110
2978	Spain	29	100	62
2982	Spain	30	100	121
3080	Mexico	30	100	115
3015	Hungary	30	100	65
VNIIMK 8883 susceptible control	Russia	30	100	115

* - number of broomrape specimens per one affected plant

On the basis of the obtained non-affected forms from all the studied collections, 6 lines resistant to race G were developed using inbreeding method. Genetic control of the resistance of one of them, line RG, has been studied. In order to study the inheritance of resistance there were carried out the cross-breedings of RG with susceptible lines of sunflower of VNIIMK breeding: VK 551, VK 678 B, VK 678 A, VK 1 IMI B, VK 1 IMI A, VK 301, VK 580 and PRO2. 12 combinations of cross-breedings were obtained, 3-8 families of each hybrid. Fifty plants of each family were evaluated for resistance to broomrape. The degree of plant infestation from the F_1 families of the studied hybrid combinations is shown in Table 2.

Table 2. The degree of infestation by broomrape of families of hybrid combinations of sunflower in F_1

Hybrid combination	Number of evaluated families	Plants infested, %	The average number of broomrape tubercles per one plant, pcs.	
			affected	acccountable*
RG × VK 580	3	43,0	2	1,2
RG × VK 551	8	56,5	5,1	4,7
VK 551 × RG	6	97,5	11,5	11,4
RG × VK 301	5	26,0	1	0,2
VK 301 × RG	6	67,0	4,8	3,2
RG × VK1-imiB	7	51,0	2,3	1,4
VK1-imiB × RG	5	62,0	2,6	1,6
VK1-imiA × RG	6	74,0	3	2,2
RG × VK 678 B	8	89,0	3	2,8
VK 678 B × RG	6	88,0	3,8	3,3
VK 678 A × RG	8	83,6	4,4	3,6
PRO2 × RG	3	55,2	2,4	1,6

*accountable plant – total number of analyzed plants from the family: affected and not affected by broomrape

All hybrid combinations F_1 were affected by broomrape. Depending on the degree of infestation, sunflower plants were divided into 3 groups. The plants were considered susceptible when more than 5 tubercles or formed broomrape sprouts were found on their roots. The plants were considered resistant when no healthy tubercles or sprouts were found on their roots, but there were numerous necroses of cells in the area of broomrape penetration and dead tubercles. Sunflower plants having five and less broomrape tubercles on the roots have been identified as genotypes with incomplete resistance.

On the basis of their damage to the plants of each family they have showed a segregation to the unaffected and affected to a small extent in comparison with the severe lesion of the control susceptible genotype. The minimum percentage of affected F_1 plants (RG × VK 301) averaged 26. The average number of broomrape tubercles per the affected plant was 1 piece, per the accountable plant it was 0.2 pieces. The maximum percentage of affected plants of F_1 hybrid (VK 551 × RG) averaged 97.5. The average number of broomrape tubercles per the affected plant was 11.5 pieces, per the accountable plant it was 11.4 pieces. But it should be noted that this combination of cross-breeding is uncharacteristic in the general number of hybrids in terms of the number of affected plants and the number of broomrape tubercles. In general, the average number of broomrape tubercles per the affected plant did not exceed 5.1 pieces, and per the accountable plant was 4.7 pieces. The obtained data indicate an incomplete dominance of the resistance trait to race G of broomrape of the line RG in F_1.

To study the influence of the reciprocal effect and the dependence of the resistance on the genetic plasma of the susceptible parent line, reciprocal cross-breedings of the line RG with sunflower lines VK 551, VK 678 B, VK 1 IMI B, and VK 301 (Table 3) were analyzed. 8 combinations of cross-breeding were received, 5-8 families of each hybrid. Fifty plants of each family were evaluated for resistance to broomrape.

Table 3. The degree of infestation by broomrape of families of reciprocal hybrid combinations

Hybrid combination	Number of evaluated families	Plants infested, %	The average number of broomrape tubercles per one accountable plant, pcs.
RG × VK 1IMI B	7	51	1,4
VK 1 IMI B × RG	5	62	2,3
SSD_{05}		11,14	1,09
RG × VK 678 B	8	89	2,80
VK 678 Б × RG	6	88	3,30
SSD_{05}		3,72	0,89
RG × VK 551	8	56,5	4,7
VK 551 × RG	6	97,5	11,4
SSD_{05}		11, 91	7,5
RG × VK 301	5	26	0,2
VK 301 × RG	6	67	3,2
SSD_{05}		16,01	3,3

In terms of the "the percentage of infested plants" and "the average number of broomrape tubercles per plant," SSD_{05} was calculated for all reciprocal cross-breeding. Differences in these characteristics were unreliable at the 5% significance level for the susceptible parental lines of VK 1 IMI B and VK 678 B. Consequently, the presence of the reciprocal effect and the dependence of resistance on the genetic plasma of susceptible parent lines participating in hybrid combinations were not proved. For the other two lines VK 551 and VK 301, differences in “the percentage of infested plants” are valid at a 5% significance level. This indicates the presence of a maternal effect in these genotypes.

The progeny F_2 and BC_1 were developed in the field conditions and were evaluated in the greenhouse for resistance and susceptibility to race G of broomrape with artificial infection. In F_2 populations, the segregation into 3 phenotypic classes was observed: resistant, slightly affected (intermediate class), and susceptible in a ratio of 1:2:1 (χ^2 = 1.40- 4.57, P=0.10-0.50) (Table 4).

Table 4. The inheritance of resistance of sunflower to the race G of broomrape in F_2 in cross-breedings of resistant line RG with susceptible lines

Cross-breeding	Number of plants, pieces			Expected segregation ratio	χ^2	df	P
	resistant	interme-diate	suscep-tible				
RG × VK 678 B	26	65	35	1:2:1	1.40	2	0.50-0.30

RG × VK 1 IMI B	25	81	38	1:2:1	4.57	2	0.20-0.10

In BC_1, the segregation into 2 phenotypic classes was observed: resistant and affected to a small extent and susceptible and affected to a small extent in a ratio of 1:1 (χ^2 = 0.03- 0.24, P=0.70-0.90) (Table 5).

Table 5. The inheritance of resistance of sunflower to the race G of broomrape in BC_1 in cross-breedings of resistant line RG with susceptible lines

Cross-breeding	Number of plants, pieces			Expected segregation ratio	χ^2	df	P
	resistant	interme-diate	suscep-tible				
(VK 678 B × RG) × VK 678 B	0	16	17	1:1	0.03	1	0.90-0.80
(VK 1 B × RG) × VK 1 B	0	12	11	1:1	0.04	1	0.90-0. 80
(VK 680 B × RG) × RGI	55	59	0	1:1	0.14	1	0,70
(PRO2 × RG) × RG	36	32	0	1:1	0.24	1	0.70

The actual segregations of F_2 and BC_1 corresponded to the monohybrid inheritance model with incomplete dominance of the trait.

Inheritance of resistance to race G in the line of sunflower RG differs from the type of inheritance in sunflower samples studied by other researchers. In the line AO-548, two independent dominant genes control genetic resistance to the population of race G of broomrape from Romania [7]. The line developed as a result of interspecies cross-breeding of cultivated sunflower with wild sunflower (*H. divarticatus*) is resistant to race G and its resistance is controlled by one recessive gene $or_{ab\text{-}vl\text{-}8}$ [8]. Velasco *et al.* [9] found that resistance to race G of broomrape in *H. debilis* subsp. *tardiflorus* in cross-breedings with cultivated sunflower is controlled by the dominant allele of one gene.

The other 5 sunflower lines that we have developed and which are not affected by race G are in the process of a hybridologic analysis to determine the genetic control of their resistance. The combination of different genes of resistance to the same race of broomrape in one sunflower genotype and the cultivation of hybrids carrying genes that control different mechanisms of resistance could contribute to the long-term resistance of the crop to the parasite.

Conclusion. Thus, 6 sunflower lines of RG line resistant to race G were developed. It is established that the resistance of one of them (RG line) is inherited monogeneuosly with incomplete dominance of a trait. In cross-breeding, the presence of the reciprocal effect and the dependence of resistance on the genotype of some susceptible parental line are established. There is no evidence of a reciprocal effect for the VK 1 IMI B and VK 678 B lines. Five non-affected lines of another origin are in the process of hybridologic analysis to determine the genetic control of their resistance.

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